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Non-destructive determination of quality traits of cashew apples (*Anacardium occidentale*, L.) using a portable near infrared spectrophotometer

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The prediction of cashew apple quality by non-destructive analysis using a portable (100 g), near infrared spectrophotometer has been investigated. The quality parameters of interest were: soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio and ascorbic acid (AA). Partial least squares calibration models were developed using the spectral range from 950 nm to 1600 nm available with the portable spectrophotometer, with a spectral resolution of 10 nm. The model was validated using an independent set of samples. Validation results gave root mean square error of prediction values of 0.6%, 0.04%, 2.7 and 27 mg 100 mL^{-1} for SSC, TA, SSC/TA and AA, respectively. According to the results, this highly portable spectrometer can be used to monitor and control cashew apple postharvest quality, and may constitute an important tool for genome wide selection in cashew breeding programmes.

Keywords: cashew apple, Micro-NIR, non-destructive method, plant breeding

Introduction

The cashew tree is productive during the drought period, contributing to employment generation and income in rural areas, especially in North-east Brazil. There are several initiatives from researchers in the agro-business to improve the quality of cashew apples and their postharvest characteristics. These efforts require reliable tools to evaluate, at high speed and with representative sampling, the principal quality parameters of cashew apples, assisting cultivar selection programmes.

Cashew apple phenotyping for genetic breeding programmes based on genome wide selection (GWS), in general, uses the same quality traits investigated in this work: soluble solid content (SSC), total acidity (TA), the ratio of SSC/TA and ascorbic acid (AA).[1–3] These variables are relevant for the selection of new clones of cashew apple with higher nutritional quality, attractive sensorial characteristics (appearance and taste) and extended shelf life.

Industrial applications have generated the need for low-cost and non-destructive quality control methods. This requirement has increased the interest in near infrared (NIR) spectroscopy, and led to the transformation from large to small and lightweight tools that enable new applications to be performed

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in situ at an industrial level.[4] Portable NIR instruments are used in many applications in the agro–food industry, particularly for the determination of solid soluble content, total acidity and firmness.[4–9]

Recently, it has become possible to acquire very compact and low-cost NIR spectrophotometers weighting about 100 g, powered through a USB port of a laptop microcomputer.[10] This type of instrument presents, besides its inherent portability, potential to extend the use of the NIR technology to small farms, producers and/or their cooperatives, multiplying the advantages of the non-destructive technology by improving the representativeness of data collection during plant selection projects. However, there are few reported evaluations of the actual performance of this type of instrument for concrete problems associated with fruit and plant selection.

This study aimed to investigate the capability of a portable NIR spectrometer as a useful, robust, low-cost and non-destructive alternative for the prediction of cashew apple quality parameters. Additionally, this work contributes to the evaluation of NIR technology applied to the determination of quality parameters of cashew apples, as the specialised literature is silent on this topic.

Materials and methods

Instrument

A portable NIR spectrophotometer (Micro-NIR 1700, Viavi, Milpitas, CA, USA) was employed throughout. The instrument operates in the 950–1600 nm range, with a spectral resolution of 10 nm, dimensions of 45 mm diameter × 42 mm high, a light weight of 100 g, is robust with no moving parts and is of relatively low cost. The instrument uses two small tungsten radiation sources to illuminate samples for reflectance measurements, and employs a continuous monochromator based on a linear variable filter (LVF), which is a band-pass filter coating, wedged in one direction and deposited over a sensor array (InGaAs) containing 126 elements. Since the centre wavelength of the bandpass is a function of the coating thickness, the wavelength transmitted through the filter, reaching a given sensor, will vary linearly in the direction of the wedge. The spectrophotometer is connected through an USB interface to a notebook computer running proprietary software for the acquisition of diffuse reflectance spectra of cashew apples.

The controlling parameters for spectral data acquisition were set at 50 ms integration time and averaging of 50 scans. The reference spectra for absorbance/reflectance calculation was obtained from a piece of Spectralon®, and renewed every hour, as was the dark signal, obtained by pointing the measurement window of the instrument to the ambient environment.

Cashew apple samples

One hundred and twenty-nine cashew apple samples, from nine varieties of early dwarf clones (*Anacardium occidentale*, L.), were randomly harvested, at different stages of maturation, from an Embrapa experimental orchard located in Pacajús (S4°10′22″; O38°27′39″), Ceará, Brazil, in October and November 2013. Eight clones were used in this work with different skin colours: yellow (CCP 06, 20 samples and Embrapa 50, 20 samples), red (BRS 189, 20 samples, BRS 265, 10 samples and CCP 1001, 10 samples), orange (CCP76, 19 samples and CCP009, 10 samples) and yellow-orange (BRS 226, 20 samples).

All fruit samples were sanitised and allowed to stabilise to room temperature (20°C ± 2°C) before NIR spectra were recorded. For each sample, the representative spectrum was taken as the average of three spectra: one spectrum obtained in each fruit region of the apex (Region 1), the middle (Region 2) and the base (Region 3). This was done to achieve better representativeness of the cashew apple inner content. According to unpublished data from our laboratory, Postharvest Embrapa, the highest variation of cashew apple composition occurs along the latitudinal direction, increasing from the apex to the base. This specific variation of the cashew apple was important in defining the measurement protocol adopted.

Multivariate regression models

The Unscrambler software package (version 10.3, CAMO AS, Oslo, Norway) was employed for data treatment and modelling. Pre-processing of the spectra set frequently improves the performance of the calibration models and, consequently, the quality of predictions. Different treatments were applied to raw NIR spectra, namely multiplicative scatter correction (MSC), standard normal variate (SNV) and first derivate Savitzky–Golay (window size five points, second order polynomial).[11]

The calibration models were developed and validated considering the *Standard Practices for Quantitative Infrared Multivariate Analysis*.[12] The performance of the model was certified by an external independent validation sample set. The calibration and validation sets were selected based on the X–Y (spectra × quality parameter) relationship by making a preliminary partial least squares (PLS) model for each parameter of interest and applying the Kenard–Stone algorithm to the values of the PLS scores of the samples. Around 60% of the samples spectra were selected and used to construct the NIR calibration models, while the remainder were used for external validation of the models. Outliers were identified and removed from the data sets based on their high leverage values, their residue, considering the preliminary models constructed.

Quality parameters determination by reference methods

The juice of the samples was extracted by mechanical crushing. SSC was estimated by placing a drop of juice on the measurement area of a digital refractometer (model PR-101, Atago, www.atago.net). TA was expressed as % ($m\,v^{-1}$) malic acid after titrating 1.0 mL aliquot of the juice with standard 0.1 $mol\,L^{-1}$ NaOH solution, using phenolphthalein as indicator. AA was determined by titrating a 1.0 mL aliquot of 1 : 50 diluted juice with 2,6-dichlorophenolindophenol reagent.[13]

Results and discussion

The raw, average reflectance spectra of cashew apples are shown in Figure 1(A). The multiplicative signal correction (MSC) pre-treatment reduces the scatter variability among the spectra, as depicted in Figure 1(B). The main absorbance peaks can be found at 900, 970, 1180 and 1450 nm, with a shoulder close to 1340 nm. The peaks at 970 nm and 1450 nm (OH stretch first overtone) can readily be assigned to water, while absorbance signals around 900 nm and 1180 nm are due to CH stretches (third and second overtone, respectively), due to carbohydrates and other organic species present in cashew skin and pulp. The shoulder at 1340 nm is probably due to CH as well, but this is more likely to represent the cellulose content of the sample.[14]

Regression models were constructed using several pre-treatments besides that of MSC described above. However, MSC shows the best performance based on the lower root mean square error of prediction (*RMSEP*) values obtained after external validation of the models. Table 1 shows the PLS modelling results obtained using only MSC as the spectral pre-processing method. The external validation results and standard errors of the reference (laboratory) methods (*SEL*) are shown in Table 2. *SEL* represents the repeatability of the reference method, and is expressed as the standard deviation of six replicates analysed by the same analyst.

We compared our results with those previously reported in the literature for other types of fruit also obtained by employing portable NIR instruments and similar regression models. For example, an apple model to determination SSC with *RMSECV* 0.72, R^2 0.77 and a strawberry model with *RMSECV* 0.66.[15,4] The *RMSEP* produced by the models constructed are always higher than the respective *SEL*. As expected, the models show an analytical performance inferior to the reference method. The difference may be attributed mainly to deficient sampling, which, even when made by averaging the data from three regions, cannot produce a spectrum fully representative of the inner content of the fruit.

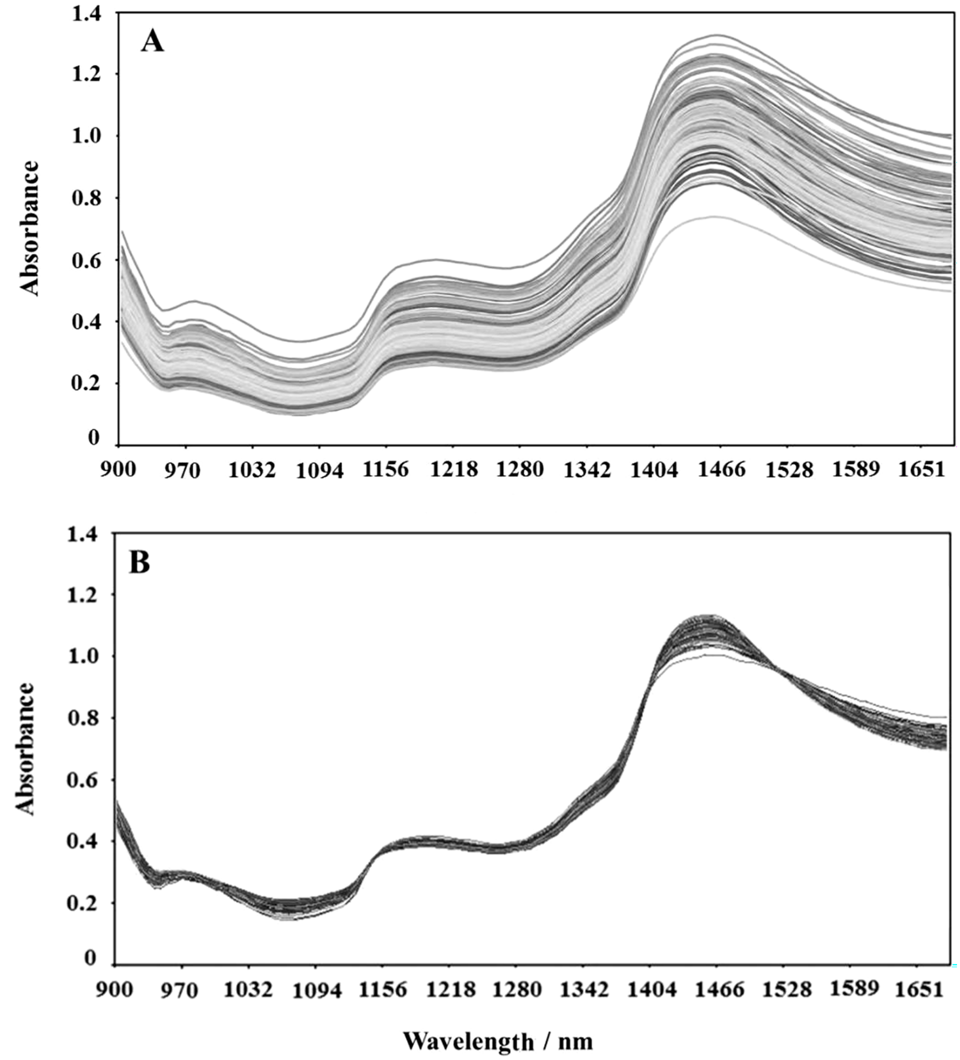

Figure 1. (A) Raw reflectance spectra of 129 cashew apple samples obtained using the portable NIR spectrophotometer. (B) The same spectra set after pre-treatment with MSC.

Table 1. Summary of the calibration and cross-validation results for SSC (%), TA (expressed as % malic acid), SSC/TA and AA (mg 100 mL^{-1}) obtained by the optimised PLS models using MSC as pre-processing algorithm.

	Number of samples (*N*)	Mean (±*SD*)	Range	Calibration			Full cross-validation	
				R^2	*RMSEC*	*LV*	R^2	*RMSECV*
AA	83	245 (47)	158–380	0.69	17	8	0.43	24
SSC	77	12.2 (1.2)	9.8–15.6	0.78	0.48	7	0.69	0.57
TA	77	0.44 (0.11)	0.27–0.94	0.57	0.06	7	0.40	0.07
SSC/TA	84	29.6 (7.2)	17.5–44.1	0.61	4.3	5	0.54	4.7

LV = optimised number of latent variables

Table 2. Summary of the results of the validation by external set of samples for SSC (%), TA (expressed as % malic acid), SSC/TA and AA (mg 100 mL^{-1}) obtained by the optimised PLS models using MSC as pre-processing algorithm and *SEL*.

	Number of samples (*N*)	Mean (±*SD*)	Range	External validation					*SEL*
				$r^2_{(max)}$	r^2	*RMSEP*	*LV*	Bias	
AA	46	244 (46)	172–307	0.85	0.67	27	8	4.2	14
SSC	52	12.0 (1.1)	10.4–13.7	0.98	0.57	0.59	5	0.06	0.13
TA	52	0.45 (0.08)	0.35–0.59	0.73	0.50	0.04	7	−0.01	0.03
SSC/TA	45	26.7 (5.4)	17.4–1.9	0.77	0.55	2.7	5	0.77	1.9

It is relevant to know if there is a significant variability among the sampling regions. This may allow the possibility to identify one specific region for measurement, which is able to represent the whole fruit, and, at the same time, reveal some information on the distribution of the different chemical species associated with the quality traits along the fruit. In order to carry out this study it was assumed that the regression models constructed with the average spectra are representative of the cashew apple composition with an accuracy represented by the estimated errors of the validated multivariate models. Then, the spectra collected from the three sampled regions were individually presented to the models in order to produce three sets of predicted values, one for each sampled region of the fruit.

The significant test was performed on the bias values admitting that a non-significant bias at 5% probability level (t calculated is less that the critical t value for $\lim_{N\to\infty} t = 1.960$.The spectra sets were of the same samples employed to validate the models constructed with spectral data averaged over the three sampling regions. The bias of each of the three groups of results was evaluated for their significance according using the equation:

$$t = (|\,\text{bias}\,|)\times\sqrt{N}\ /\ SEP \tag{1}$$

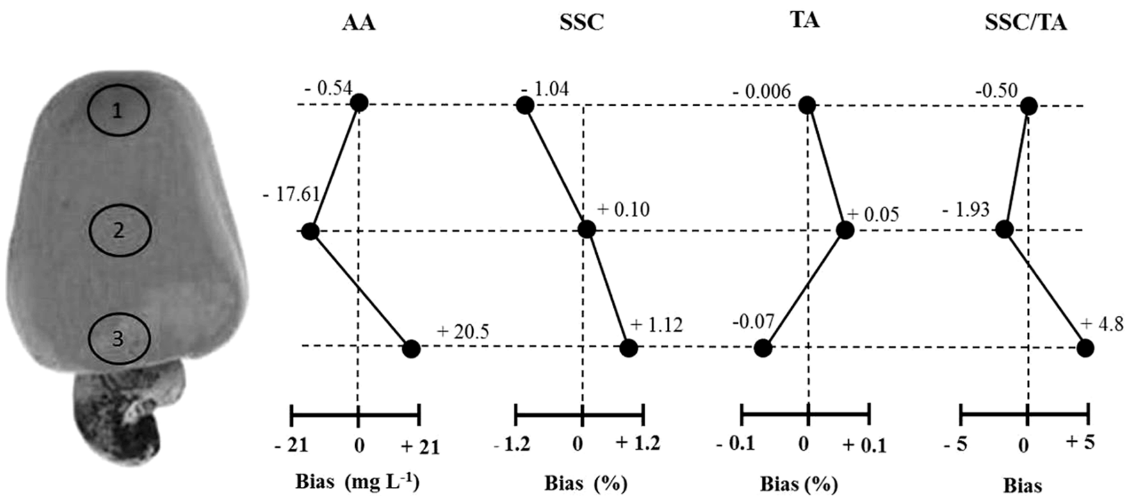

Figure 2. Bias for external validation obtained by predicting cashew apple quality traits using the spectra of each region of the fruit and regression models constructed using the average spectra of the three regions.

Significant bias means that the results obtained by using the spectra collected in one specific region differ significantly and systematically from the results obtained by using the data averaged over the three regions. The bias signal shows if the difference is positive or negative (estimates are higher or lower than the reference values of the property). Figure 2 shows the estimated bias for the four quality properties evaluated in this work as a function of the sampling region.

The result revealed that, in fact, the best representativeness was achieved by averaging the spectra acquired in the three sampled regions of the fruit. This does not mean that the proposed sampling protocol used in this work generates data that are the most representative of the whole fruit. As mentioned, the protocol was designed to facilitate and expedite the spectral data acquisition from the fruit while it is on the plant, where access may only be available to one side of the fruit. At the same time, the bias values define a distinct pattern for each property along the fruit, showing the difficulty of establishing one single sampling protocol to achieve the best representativeness for all properties.

The statistical test showed that AA and TA could be determined by using only the spectra collected from region 1. On the other hand, large positive systematic errors were found in the determination of SSC. For the ratio SSC/TA, perhaps compensation between systematic errors also indicates that region 1 is able to evaluate this quality property, though the calculated t-value is relatively closer to the threshold.

Conclusion

It was demonstrated that the portable NIR spectrophotometer, the spectral sampling protocol and the PLS models resulted in an analytical method with performance comparable with other portable NIR instruments for the prediction of four principal quality traits of intact cashew apples. Because this is the first work in the literature dealing with NIR spectroscopic analysis of cashew apples, the comparison had to be made with results obtained when the technology was applied to other types of fruit. The instrument used in this work is exceptionally portable and of low cost, allowing for its use in shelf quality evaluation and for in-field monitoring.

The proposed fruit sampling protocol is far from ideal, as shown by the significant bias found for the values of the quality traits around the cashew apple. However, spectra obtained by averaging data from three sampling locations produced reliable regression models suitable to determine bulk values of the quality traits evaluated in this work. The accuracy here for cashew apples is comparable to that of other previous work and is sufficient to encourage cashew producers to make use of the method. In particular, the values of *RMSEP* obtained for the four quality characteristics of cashew apple, though greater than the errors of the reference methods (*SEL*), qualify the proposed method to help in programmes of genome wide selection of cashew cultivars for commercial purposes. For this purpose, the ease of measurement of the intact fruits prior to harvest and the better representativeness achieved by including more samples in the quality monitoring represent significant advantages of the use of portable NIR spectroscopy in the cashew apple industry.

Because the cashew apple is a non-climacteric fruit, further studies will be carried out to evaluate if the portable NIR spectrophotometer can follow the maturation process and determine the best harvest time in the field.

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